REMARKS

The specification has been amended to correct the "hand" symbols and "lined page" symbols that inadvertently appeared in the printed version. The Brief Description of the Drawings has been amended to reflect the changes to Figures 6 and 7 as recommended by the Examiner.

Claims 1-46 are pending in the application. By this amendment, claims ____ are amended. The amendments are for clarity and are supported throughout the Specification. Support for specific amendments are described in the sections where they are discussed. No new matter is added.

Figures 6 and 7 have been corrected to refer to each embodiment by frame, as suggested by the Examiner. Annotated pages of Figures 6 and 7 are attached.

Objections to the Specification

The specification was objected to as including some unclear symbols. The specification is been amended to correct these clerical errors. All "hand" symbols are replaced by the Greek alphabet beta (ß) and all "page" symbols are replaced by the Greek alphabet delta (Δ).

Rejections Under 35 U.S.C.§ 112

(i) Claims 24-33, and 34-46 are rejected under 35 U.S.C. § 112, first paragraph as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Specifically, the Examiner states that the specification does not provide any structures of an agent that alters native DNA methylase activity.

Applicants respectfully traverse. Paragraph 202 (p. 54) describes functions and structures that characterize the "agents" specified in the claims. Typical structures of agents are disclosed including anti-sense RNA, polypeptide and chelator. Applicants submit that determining structures of e.g., anti-sense RNA is well-known in the art and thus the term "agent" is enabled by the Specification.

(ii) Claims 24-33 are rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for a method of stimulating an immune response against a pathogen, does not reasonably provide enablement for stimulation of a protective immune response that can prevent or treat infection caused by the pathogen when dam gene activity is altered. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Applicants respectfully traverse. The Specification clearly illustrates that immunization with Dam$^-$ bacteria hinders the growth of virulent bacteria in systemic tissues. ("Dam$^-$ mutants are unable to cause disease but are able to elicit a full-protective immune response;" *see* paragraph 274, p. 82 and Fig. 7). The data in Figures 7A-7D shows that following challenge with virulent organisms "the (Dam-negative) immunized mice have the ability not only to inhibit the growth of these virulent organisms, they are capable of clearing them from both mucosal and systemic tissues."

The Examiner raises concerns about the reversion of Dam negative mutations based on Torreblanca and Kupcella. However, as shown in Fig. 1, these are severely deficient in colonization of deeper tissue sites (liver and spleen) (Example 1). Dam$^-$ mutants of *S. typhimurium* are also less cytotoxic to M cells, are deficient in epithelial invasion, and display defects in protein secretion. (*see* para 274, p. 82). Since it is possible to test for Dam-negativity prior to immunization, the likelihood of infection is minimal.

The Examiner's reliance on Ellis and Boslego for problems with protective immunity does not address the present invention. The Specification discloses immunizing with Dam$^-$ strains of a bacterium in order to generate protection against virulent bacteria that include other unrelated strains. Ellis and Boslego relate to direct immunization by an immunogen for protection against that immunogen. The present invention relates to general protection following administration of a Dam$^-$ bacteria.

The Examiner also seeks the identity of specific genes that alter Dam activity and induce protective immunity. As discussed above, the specification discloses the protection against virulent bacteria by administration of a bacterium that has altered DNA adenine methylase activity. The

Examiner has not pointed to any reason why one would expect different results from different methods for alteration of Dam activity. The key feature is altered Dam activity. Therefore, Applicants submit that the specification enables the claimed invention.

(iii) Claims 1-18, 20-23, 25-28 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1 has been amended to specify "reducing" virulence which has antecedent basis in the preamble.

In response to the Examiner's concern over an apparent contradiction between claims 2-4 and 6 (reducing Dam) and claims 5 and 7 (increasing Dam), insofar as they both reduce virulence of bacteria, Applicants refer to paragraph [0080] on pages 20-21 which states that "[t]he level of activity can be *decreased or increased and either will render the cell substantially less virulent* as compared to an equivalent, unmodified, wild-type cells." (emphasis added). The same paragraph explains that the cause of the discovered phenomenon is that "by altering the level ... and/or the activity ... of Dam in a cell the balance of the cell is upset." The reduction of pathogenicity of a pathogenic bacteria by **increasing or reducing** Dam activity is also discussed in paragraph [00110] on page 27 of the Specification. Table 1 and para 248 (p. 70) of the Specification demonstrates that both Dam overproducer and Dam$^-$ strains similarly reduce bacterial virulence "suggesting that precise levels of Dam methylase are required for full virulence." Also Example 8 shows Dam overproducing strains of *Yersinia* and *Vibrio* are avirulent.

(iv) Claims 1-18 are rejected under 35 U.S.C. § 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps.

In response, Applicants amend claim 1 to include the step of "providing a virulent bacteria having a DNA methyltransferase (Dam) activity."

Applicants submit that the additional step deemed necessary to be specified by the Examiner ("determining the native level of DNA methyltransferse activity") would result in an unnecessary

limitation to the claim. Determination of the native level of DNA methyltransferse activity may be a necessary step in identifying the agent, however, once an agent is known to possess such a property the only relevant activity is "reducing virulence of the bacteria."

(v) The Examiner expresses concern about the antecedent basis of the term "Dam enzyme" in claim 8. In response, Applicants amend claim 8 to further specify that "the Dam enzyme is responsible for the DNA methyltransferase (Dam) activity in the bacteria." The "DNA methyltransferase (Dam) activity in the bacteria" is specified in claim 1 from which claim 8 depends.

(vi) The Examiner objects to the terms reduces or increases (in the alternative) Dam activity in order to reduce virulence in claims 2-18, 20-23, 25-28, 37-38 as contradictory and therefore indefinite.

As discussed above, Applicants refer to paragraph [0080] on pages 20-21 which states that "[t]he level of activity can be *decreased or increased and either will render the cell substantially less virulent* as compared to an equivalent, unmodified, wild-type cells." (emphasis added). Table 1 and para 248 (p. 70) of the Specification demonstrate the similar effects of increased or reduced Dam activity..

(vii) The terms "decreases" and "normal" in claim 9 are found to be indefinite as "normal" is not defined in the Specification.

In response, Applicants amend claim 9 to specify that binding of the agent to a native sequence of the bacteria "decreases expression of a Dam *gene* below a *native* level" (emphasis added). Applicants submit that paragraphs 105-106 (p. 26), 202-205 (pp. 54-55) provide support for this amendment. Native (or wild type) level of Dam activity in a wild type bacteria is described throughout the Specification and is specified in independent claim 1 from which claim 9 depends. Claims 9 and 10, as amended specifies a level of activity that is lower (or higher) than that in a native pathogenic bacteria.

(viii) Claims 11-18 are objected to for lacking antecedent basis for the term "pathogenic bacteria."

In response, Applicants amend claim 1, from which claims 11-18 depend, to specify a method directed to a "method of reducing bacterial virulence of a pathogenic bacteria."

(ix) Claims 20 and 25 are objected to for reciting "expression of Dam" while the base claim recites a Dam enzyme activity.

In response, Applicants amend claims 20 and 25 to specify "expression of a Dam gene responsible for the DNA methyltransferase activity" specified in the base claims 19 and 24 respectively.

(x) Claims 21 and 26 are objected to for reciting "a Dam interaction site" and it is unclear whether Dam refers to a gene or enzyme activity.

In response, Applicants amend claims 21 and 26 to specify that the agent binds to a "Dam enzyme interaction site." Support for this amendment can be found *inter alia* in paragraphs 109 (p. 27), and 207 (pp. 55-56).

(xi) Claim 24 is amended to recite "treating a *pathogenic* bacterial infection by *inhibiting proliferation of the bacteria*" (emphasis added) in the preamble to remove ambiguities as to the pathogenicity of the bacteria specified in the claim and the antecedent basis for inhibition of proliferation in the claim. Support for the amendment can be found in para 193 on page 52 of the Specification.

(xii) Claims 27 and 28 are amended to recite "a pathogenic bacterial infection" in the preamble to remove ambiguities as to the pathogenicity of the bacteria specified in the claim.

Claims 27 and 28 are further amended for clarity to delete references to "inhibiting virulence" and instead specifying "inhibiting proliferation" of the bacteria in accordance with the base claim 24.

(xiii) Claim 32 is amended to recite "method of treating bacterial infection *in an individual* comprising administering *to the individual* an agent" provide the essential missing steps. (emphasis added).

(xiv) Claims 36-38 and 40 are amended to recite "compound" instead of "agent " in conformation with claim 34 from which they depend.

(xv) Claim 34 is amended to recite "pathogenicity of a bacteria" instead of "bacterial pathogenicity." This re-wording provides antecedent basis for claims 39-40.

(xvi) Claims 17 and 45 are amended to recite dependency from claims 1 and 34 respectively instead of claims 12 and 40 as originally filed. Thus, claims 17 and 45 no longer broaden the scope of the claims from which they depend. The language of claims 12 and 40 is incorporated in amended claims 17 and 45 except that the two sets of claims recite independent and non-overlapping Markush groups. No new matter is added.

Rejections Under 35 U.S.C. § 102

(i) Claims 1-2, 11-15, 17-18, and 19 are rejected under 35 U.S.C. § 102(e) as being anticipated by Vermeulen et al (US Pat. No. 5,872,104).

Applicants respectfully traverse. Vermeulen does not disclose reduction of virulence by inhibition of methylation. Vermeulen discloses "increasing the effectiveness of MLS antibiotics" (col. 5 lines 10-24) based on the contemplation that "methylation ... plays a role in all mechanisms of resistance of a microorganism to an antimicrobial agent." (col. 3, lines 8-13). A bacteria can retain it's virulence or pathogenicity while being susceptible to antibiotics and Vermeulen does not teach or suggest methods for reducing the "virulence" or "pathogenicity" of a "pathogenic bacteria" as specified in independent claims 1 and 19. Instead Vermeulen claims (claim 53 *et seq.*) administering "to an animal with a non-viral microorganism infection *a therapeutically effective amount of an antimicrobial agent* in combination with a therapeutic amount of a methylation inhibitor," i.e., an *antibiotic* in combination with a methylase inhibitor. (emphasis added)

Further, Vermeulen teaches use of general methylase inhibitors and not DNA adenine methylase (Dam) inhibitors as specified in independent claims 1 and 19. For example, the Examiner cites the methylase inhibitor "sinefungin" as an example of an agent ("especially col. 46, lines 5-17" Office Action page 12, section 15.). Applicants refer to paragraph 00210 of the Specification (p. 57) where the inventors clearly distinguish the agents of the present invention from the type of agents represented by sinefungin: "However, because sinefungin would block all DNA methylases including the mammalian cytosine methylase that require SAM as methyl donor, *this drug would not be useful* as a chemotherapeutic agent against bacteria." (emphasis added)

Since Vermeulen does not teach or suggest the agents specified in independent claims 1 and 19, and claims 2, 11-15, and 17-18 depend therefrom, Applicants respectfully request withdrawal of this ground for rejection.

(ii) Claims 1, 5, 7, 10, 19, 22 (increased level of activity) are rejected under 35 U.S.C. § 102(b) as being anticipated by Blyn et al.

Applicants respectfully traverse. Blyn does not teach or suggest reduction of *virulence* or *pathogenicity* by altering Dam activity as specified in independent claims 1 and 19. Blyn teaches bacteria which have been transformed with a plasmid carrying a dam gene, any resulting effect on virulence or pathogenicity is not discussed.

In addition, Blyn does not teach or suggest "contacting" or "administering to" the bacteria an agent that alters the bacteria's native level of DNA methyltransferase (Dam) activity thereby altering the bacteria's native level of methylation. The Examiner cites page 4049, col. 2 of Blyn where the bacteria are transformed with plasmids carrying a Dam gene. it is well-known in the art that merely "contacting" bacteria with a plasmid does not transform the bacteria and additional procedures are necessary for transformation.

Since Blyn does not teach or suggest each and every element of independent claims 1 and 19 and claims 5, 7, 10, and 22 depend therefrom, Applicants respectfully request withdrawal of this ground for rejection.

In light of the amendments and arguments set forth above, Applicants earnestly believe that they are entitled to a letters patent and respectfully request the Examiner to expedite prosecution of this patent application to issuance. Should the Examiner have any questions, the Examiner is encouraged to telephone the undersigned.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 03-1952 referencing docket no. 220002060725. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: February 27, 2004

Respectfully submitted,

By_____
Shantanu Basu
Registration No.: 43,318
MORRISON & FOERSTER LLP
755 Page Mill Road
Palo Alto, California 94304
(650) 813-5995